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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 C.F.R. § 1.53(c).

	Docket Number	14114.0378U1	Type a Plus Sign (+) inside this box	+

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TITLE OF INVENTION (500 characters max)

RAb9 AND ITS USES THEREOF RELATED TO INFECTIOUS DISEASES

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ENCLOSED APPLICATION PARTS (*Check All That Apply*)

☒	Provisional Application Title Page	*Number of Pages*	[1]
☒	Specification (includes Description, Claims, & Abstract)	*Number of Pages*	[24]
☐	Drawing(s)	*Number of Sheets*	[0]
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METHOD PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (*Check One*)	
☐ Applicant claims small entity status. See 37 CFR § 1.27. ☒ A Credit Card Payment Form PTO-2038 is enclosed to cover the filing fees. ☐ A check or money order is enclosed to cover the filing fees. ☐ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number __________. ☒ The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 14-0629.	**FILING FEE AMOUNT** $ 160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes. The name of the U.S. Government agency and the Government contract number are: Centers for Disease Control and Prevention

Respectfully submitted,

Signature [signature] **Date** 2/24/04

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Attorney Docket No. 14114.0378U1

UTILITY PATENT - PROVISIONAL FILING

<u>PROVISIONAL</u> APPLICATION FOR LETTERS PATENT

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Thomas W. Hodge, Natalie J. Morey, Donald Rubin, Michael W. Shaw, and Anthony Sanchez, residing respectively at 9115 Twelvestone Drive, Roswell, Georgia 30076, 3138 Caldwell Road NE, Atlanta, Georgia 30319-2918, 112 Harding Hill Lane, Nashville Tennessee 37215, 2614 Willow Cove, Decatur, Georgia 30033-2200, and 1717 Red Fox Run, Lilburn, Georgia 30047 have invented new and useful improvements in

RAb9 AND ITS USES THEREOF RELATED TO INFECTIOUS DISEASES

for which the following is a specification.

RAB9 AND USES THEREOF RELATED TO INFECTIOUS DISEASE

FIELD OF THE INVENTION

The present invention relates to a cellular protein, Rab9, Rab9 nucleic acid sequences and Rab9 proteins encoded by these sequences that are involved in viral infection or are otherwise associated with the life cycle of a virus. The invention also relates to modulators of Rab9 that are involved in viral infection or are otherwise associated with the life cycle of a virus.

DETAILED DESCRIPTION OF THE INVENTION

The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein.

Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that this invention is not limited to specific nucleic acids, specific polypeptides, or to particular methods, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. The term "or" refers to a single element of stated alternative elements or a combination of two or more elements, unless the context clearly indicates otherwise. As used herein, "comprises" means "includes." Thus, "comprising A or B," means "including A, B, or A and B," without excluding additional elements.

Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where

said event or circumstance occurs and instances where it does not. For example, the phrase "optionally obtained prior to treatment" means obtained before treatment, after treatment, or not at all.

As used throughout, by "subject" is meant an individual. Preferably, the subject is a mammal such as a primate, and, more preferably, a human. The term "subject" includes domesticated animals, such as cats, dogs, etc., livestock (for example, cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (for example, mouse, rabbit, rat, gerbil, guinea pig, etc.).

Several host nucleic acid sequences involved in viral infection have been identified using gene trap methods. The identification of these host sequences and their encoded proteins permits the identification of sequences that can be targeted for therapeutic intervention. One of the host nucleic acid encodes Rab9, a cellular protein that is a Ras oncogene family member. Rab9 is a host protein involved in infection by pathogens (including, but not limited to, viruses, bacteria and fungi) that use similar pathways for morphogenesis of infectious particles.

The term Rab9 includes any Rab9 gene, cDNA, RNA, or protein from any organism that can transport late endosomes to the trans-golgi and function as a ras-like GTPase. In some examples, Rab9 is involved in lipid raft formation.

Examples of native Rab9 sequences include, but are not limited to GenBank Accession Nos. BC017265.2 and NM_004251.3 (cDNA) as well as P51151 and AAH17265 (proteins). The nucleic acid sequences and protein sequences provided under the GenBank Accession Nos. mentioned herein are hereby incorporated in their entireties by this reference. In one example, a Rab9 sequence includes a full-length wild-type (or native) sequence, as well as Rab9 allelic variants, variants, fragments, homologs or fusion sequences that retain the ability to transport late endosomes to trans-golgi. In certain examples, Rab9 has at least 80% sequence identity, for example at least 85%, 90%, 95%, or 98% sequence identity to a native Rab9. In other examples, Rab9 has a sequence that hybridizes to a sequence set forth in GenBank Accession No. BC017265.2 or NM_004251.3 and retains Rab9 activity.

Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (such as the Na+ concentration) of the hybridization buffer will determine the stringency of hybridization. Calculations regarding hybridization

conditions for attaining particular degrees of stringency are discussed in Sambrook *et al.*, (1989) Molecular Cloning, second edition, Cold Spring Harbor Laboratory, Plainview, NY (chapters 9 and 11). The following is an exemplary set of hybridization conditions and is not limiting:

Very High Stringency (detects sequences that share 90% identity)

Hybridization:	5x SSC at 65°C for 16 hours
Wash twice:	2x SSC at room temperature (RT) for 15 minutes each
Wash twice:	0.5x SSC at 65°C for 20 minutes each

High Stringency (detects sequences that share 80% identity or greater)

Hybridization:	5x-6x SSC at 65°C-70°C for 16-20 hours
Wash twice:	2x SSC at RT for 5-20 minutes each
Wash twice:	1x SSC at 55°C-70°C for 30 minutes each

Low Stringency (detects sequences that share greater than 50% identity)

Hybridization:	6x SSC at RT to 55°C for 16-20 hours
Wash at least twice:	2x-3x SSC at RT to 55°C for 20-30 minutes each.

As mentioned above, Rab9 is involved in viral infection. Furthermore, Rab9 is a small GTPase that traffics proteins and other molecules from the late endosome to the trans-Golgi. Therefore, other proteins involved in this trafficking pathway and/or any interaction between Rab9 and these proteins, can be involved in viral infection. Therefore, the present invention contemplates the identification of upstream and downstream Rab9 modulators that are involved in viral infection.

Identification of Rab9 modulators

The present invention provides a method of identifying a modulator of Rab9 that is involved in viral infection comprising: a) inhibiting the expression of a putative modulator of Rab9 in a cell that expresses Rab9; b) contacting the cell with a virus; and c) detecting the level of viral infection, a decrease or elimination of viral infection indicating that the putative modulator of Rab9 is a modulator of Rab9 that is involved in viral infection.

The present invention also provides a method of identifying a modulator of Rab9 that is involved in viral infection comprising: a) inhibiting the expression of a putative modulator of Rab9 in a cell that expresses Rab9; b) contacting the cell with a virus; c) detecting the level of viral infection; and d) associating the level of viral infection with the level of Rab9 and/or Rab9 activity, a decrease or elimination of viral infection associated with a decrease or elimination of Rab9 and/or Rab9 activity indicating that the putative modulator of Rab9 is a modulator of Rab9 that is involved in viral infection.

As utilized herein, "modulator of Rab9" or "Rab9 modulator" means a molecule, such as a nucleic acid or protein that interacts, either directly or indirectly with Rab9. For example, a Rab9 modulator can affect transcription of Rab9 mRNA and/or translation of Rab9 mRNA. A Rab9 modulator can also be a protein involved in trafficking proteins and other molecules from the late endosome to the trans-Golgi via interaction with Rab9. Therefore, a Rab9 modulator can be a protein that binds to, or is involved in a complex with Rab9, and is involved in trafficking proteins and other molecules from the late endosome to the trans-Golgi.

In the methods of the present invention any cell that can be infected with a virus or other pathogen, such as bacteria or fungi can be utilized. The cell can be prokaryotic or eukaryotic, such as a cell from an insect, crustacean, mammal, bird, reptile, yeast or a bacteria, such as *E. coli*. The cell can be part of an organism, or part of a cell culture, such as a culture of mammalian cells or a bacterial culture.

Examples of viruses include, but are not limited to, HIV (including HIV-1 and HIV-2), parvovirus, papillomaviruses, measles, filovirus, SARS (severe acute respiratory syndrome) virus, hantaviruses, influenza viruses (*e.g.,* influenza A, B and C viruses), hepatitis viruses A to G, caliciviruses, astroviruses, rotaviruses, coronaviruses, (for example, human respiratory coronavirus), picornaviruses, (for example, human rhinovirus and enterovirus), Ebola virus, human herpesvirus (such as, HSV-1-9, including zoster, Epstein-Bar, and human cytomegalovirus), human adenovirus, smallpox virus and hantaviruses.

For animals, viruses include, but are not limited to, the animal counterpart to any above listed human virus, avian influenza, and animal retroviruses, such as simian immunodeficiency virus, avian immunodeficiency virus, bovine immunodeficiency virus, feline immunodeficiency virus, equine infectious anemia virus, caprine arthritis encephalitis virus and visna virus.

Examples of bacteria include, but are not limited to, the following: *Listeria* (*spp.*), *Mycobacterium tuberculosis*, *Rickettsia* (all types), *Ehrlichia*, *Chylamida*. Further examples of bacteria that can be targeted by the present methods include *M. tuberculosis, M. bovis, M. bovis* strain BCG, BCG substrains, *M. avium, M. intracellulare, M. africanum, M. kansasii, M. marinum, M. ulcerans, M. avium* subspecies *paratuberculosis, Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi,* other *Salmonella* species, *Shigella* species, *Yersinia pestis, Pasteurella haemolytica, Pasteurella multocida,* other *Pasteurella species, Actinobacillus pleuropneumoniae, Listeria monocytogenes, Listeria ivanovii, Brucella abortus,* other *Brucella* species, *Cowdria ruminantium, Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydia psittaci, Coxiella burnetti,* other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae, Bacillus anthracis, Escherichia coli, Vibrio cholerae, Campylobacter* species, *Neiserria meningitidis, Neiserria gonorrhea, Pseudomonas aeruginosa,* other *Pseudomonas* species, *Haemophilus influenzae, Haemophilus ducreyi,* other *Hemophilus* species, *Clostridium tetani,* other *Clostridium* species, *Yersinia enterolitica,* and other *Yersinia* species.

The methods of the present invention can be used to target parasites. Examples of parasites that can be targeted include, but are not limited to, the following: *Cryptosporidium*, *Plasmodium* (all species), American trypanosomes (*T. cruzi*). Furthermore, examples of protozoan and fungal species contemplated within the present methods include, but are not limited to, *Plasmodium falciparum*, other *Plasmodium* species, *Toxoplasma gondii*, *Pneumocystis carinii*, *Trypanosoma cruzi*, other trypanosomal species, *Leishmania donovani*, other *Leishmania species*, *Theileria annulata,* other *Theileria* species, *Eimeria tenella,* other *Eimeria* species, *Histoplasma capsulatum, Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Penicillium marneffei*, and *Candida* species.

There are numerous methods known in the art for inhibiting or decreasing the expression of a Rab9 modulator in a cell that expresses Rab9. A decrease in expression or inhibition of expression need not be complete, as this can range from a slight decrease in expression to completely eliminating expression, such as reduction of at least 10%, at least 50%, at least 90% or even at least 99%. The Rab9 modulators of the present invention can be inhibited by knocking out a Rab9 modulator gene. Gene knockout methodology is well known in the art and can be performed via gene

trapping, site directed mutagenesis, and morpholinos. Functional deletions can also be accomplished by mutation, partial or complete deletion, insertion or other variation to a gene sequence that inhibits production of the gene product, or renders the gene product non-functional. For example, a functional deletion of a Rab9 gene or a Rab9 modulator gene in a cell results in cells having a non-functional Rab9 or non-functional Rab9 modulator which results in a cell having increased resistance to infection by a pathogen.

A Rab9 modulator can also be inhibited by decreasing or disrupting transcription and/or translation of mRNA encoding the Rab9 modulator. Methods for accomplishing this include, but are not limited to contacting the Rab9 modulator mRNA with an antisense RNA, RNAi, ribozyme, or small interfering RNA (siRNA) that recognizes the mRNA.

A Rab9 modulator can also be inhibited by contacting the Rab9 modulator in the cell with an antibody, a polyeptide, a chemical, small molecules or a drug that inhibits the interaction (for example, a binding interaction) between the Rab9 modulator and Rab9 or an interaction between a Rab9 modulator and a viral protein. cDNAs can also be used in the methods of the present invention. cDNAs can be transfected into cells to assess the effects of the proteins encoded by these cDNAs on the interaction between Rab9 and a Rab9 modulator.

Once the Rab9 modulator has been contacted with an inhibitor, the level of viral infection can be associated with the level of Rab9 and/or Rab9 activity, such that a decrease or elimination of viral infection associated with a decrease or elimination of Rab9 and/or Rab9 activity indicating that the Rab9 modulator is a Rab9 modulator that is involved in viral infection.

Viral infection can be measured via cell based assays. Briefly, cells (20,000 to 250,000) are infected with the desired pathogen, and the incubation continued for 3-7 days. The agent that inhibits the expression of the Rab9 modulator can be applied to the cells before, during, or after infection with the pathogen. The amount of virus and agent administered can be determined by skilled practitioners. In some examples, several different doses of the potential therapeutic agent can be administered, to identify optimal dose ranges. Following transfection, assays are conducted to determine the resistance of the cells to infection by various agents.

For example, if analyzing viral infection, the presence of a viral antigen can be determined by using antibody specific for the viral protein then detecting the antibody. In one example, the antibody that specifically binds to the viral protein is labeled, for

example with a detectable marker such as a fluorophore. In another example, the antibody is detected by using a secondary antibody containing a label. The presence of bound antibody is then detected, for example using microscopy, flow cytometry and ELISA.

Alternatively, or in addition, the ability of the cells to survive viral infection is determined, for example, by performing a cell viability assay, such as trypan blue exclusion.

The amount of Rab9 protein in a cell can be determined by methods standard in the art for quantitating proteins in a cell, such as Western blotting, ELISA, ELISPOT, immunoprecipitation, immunofluorescence (e.g., FACS), immunohistochemistry, immunocytochemistry, etc., as well as any other method now known or later developed for quantitating protein in or produced by a cell.

The amount of Rab9 nucleic acid in a cell can be determined by methods standard in the art for quantitating nucleic acid in a cell, such as *in situ* hybridization, quantitative PCR, RT-PCR, Northern blotting, ELISPOT, dot blotting, etc., as well as any other method now known or later developed for quantitating the amount of a nucleic acid in a cell.

One of skill in the art can also assess the effect(s) of inhibiting a Rab9 modulator on Rab9 activity by observing differences in vesicle transport from endosomes to the trans-Golgi. Methods for visualizing this process are standard in the art. For example, see Barbero et al. "Visualization of Rab9-mediated vesicle transport from endosomes to the trans-Golgi in living cells," *Journal of Cell Biology*, 156(3): 511-518 (2002), which is incorporated in its entirety by this reference as it pertains to visualization of Rab9 vesicular transport and interactions of Rab9 with other proteins (for example, Mannose 6 phosphate receptors, TIP47, Rab9 effector p40 and mapmodulin involved in vesicle transport from endosomes to the trans-Golgi). The interaction between Rab9 and other proteins can be direct or indirect. An example of a direct interaction would be the binding of Rab9 to another protein. An example of an indirect interaction would be an interaction between two or more proteins involved in vesicle transport and/or viral infection, either upstream or downstream of Rab9, that affect an activity of Rab9, but do not interact directly with Rab9.

An example of a protein that interacts with Rab9 is GDI-displacement factor (GDF). See Dirac-Svejstrup et al. "Identification of a GDI displacement factor that releases endosomal Rab GTPases from Rab-GDI," *The EMBO Journal* 16(3): 465-472

(1997). This reference is incorporated herein in its entirety as it relates to an interaction involving Rab9 and GDF.

Another example of a protein that interacts with Rab9 is Rab9 effector p40. See Diaz et al. "A Novel Rab9 Effector Required for Endosome-toTGN-Transport," The Journal of Cell Biology 138(2):283-290 (1997). This reference is incorporated herein in its entirety as it relates to an interaction involving Rab9 and Rab9 effector p40. A nucleic acid encoding RAb9 effector p40 and a Rab9 effector p40 polypeptide sequence can be found under GenBank Accession No. BC053541. These sequences are incorporated herein by this reference.

Another example of a protein that interacts with Rab9 is Rab-GDI. See Dirac-Svejstrup et al. "Rab-GDI Presents Functional Rab9 to the Intracellular Transport Machinery and Contributes Selectivity to Rab9 Membrane Recruitment," Journal of Biological Chemistry 269(22): 15427-15439 (1994). This reference is incorporated herein in its entirety as it relates to an interaction involving Rab9 and Rab-GDI. A nucleic acid encoding Rab-GDI and a RAB-GDI polypeptide sequence can be found under GenBank Accession No. NM_001493. These sequences are incorporated herein by this reference.

Another example of a protein that interacts with Rab9 is Yip3. See Sivars et al. "Yip3 caltalyzes the dissociation of endosomal Rab-GDI complexes," *Nature* 425(6960):856-859 (2003). This reference is incorporated herein in its entirety as it relates to an interaction involving Rab9 and Yip3. A nucleic acid encoding Yip3 and a Yip3 polypeptide sequence can be found under GenBank Accession No. NM_006423. These sequences are incorporated herein by this reference.

Another example of a protein that interacts with Rab9 is PIKfyve. See Ikonomov et al. "Active PIKfyve Associates with and Promotes the Membrane Attachment of the Late Endosome-to trans-Golgi Network Transport Factor Rab9 Effector p40," Journal of Biological Chemistry 278(51):50863-50871 (2003). PIKfyve interacts with the Rab9effector p40 and is thus important in the Rab9 vesicle transport mechanism. This reference is incorporated herein in its entirety as it relates to an interaction between Rab9 and PIKfyve. A nucleic acid encoding PIKfyve and a PIKfyve polypeptide can be found under GenBank Accession No. NM_015040. These sequences are incorporated herein by this reference.

Another example of a protein that can interact with Rab9 is TSG101. See Panchal et al. "In vivo oligomerization and raft localization of Ebola virus protein

VP40 during vesicular budding. *Proc Natl Acad Sci U S A*. Dec 23;100(26):15936-41 (2003). A nucleic acid encoding TSG101 and a TSG101 polypeptide sequence can be found under GenBank Accession No. NM_006292. These sequences are incorporated herein by this reference.

Another example of a protein that can interact with Rab9 is TIP47. A nucleic acid encoding TIP47 and a TIP47 polypeptide sequence can be found under GenBank Accession No. NM_005817. These sequences are incorporated herein by this reference.

Another example of a protein that can interact with Rab9 is a v-SNARE protein. A nucleic acid encoding a v-SNARE protein and a v-SNARE polypeptide sequence can be found under GenBank Accession No. XM-371718. These sequences are incorporated herein by this reference.

Another example of a protein that can interact with Rab9 is the mannose-6-phosphate receptor protein. A nucleic acid encoding a mannose-6-phosphate receptor and a mannose-6-phosphate receptor polypeptide sequence can be found under GenBank Accession No. NM_002355. These sequences are incorporated herein by this reference.

Therefore, in the methods of the present invention the expression or activity of any one or more Rab9 modulators involved in vesicle transport from endosomes to the trans-Golgi can be inhibited. These include, but are not limited to, mannose-6-phosphate receptor, a GDI-displacement factor, TSG101, Rab9 effector p40, Yip3, PIKfyve, Rab-GDI, GDF, TIP47 and mapmodulin, a v-SNARE protein, a T-SNARE protein and a guanine nucleotide exchange factor (GEF).

The present invention also provides a method of determining if a modulator of Rab9 is associated with viral infection comprising inhibiting the expression of the Rab9 modulator in a cell that expresses Rab9 and determining if viral infection is inhibited.

Viral infection can be measured via cell based assays. Briefly, cells (20,000 to 250,000) are infected with the desired pathogen, and the incubation continued for 3-7 days. The agent that inhibits the expression of the Rab9 modulator can be applied to the cells before, during, or after infection with the pathogen. The amount of virus and agent administered can be determined by skilled practitioners. In some examples, several different doses of the potential therapeutic agent can be administered, to identify optimal dose ranges. Following transfection, assays are conducted to determine the resistance of the cells to infection by various agents.

For example, if analyzing viral infection, the presence of a viral antigen can be determined by using antibody specific for the viral protein then detecting the antibody. In one example, the antibody that specifically binds to the viral protein is labeled, for example with a detectable marker such as a fluorophore. In another example, the antibody is detected by using a secondary antibody containing a label. The presence of bound antibody is then detected, for example using microscopy, flow cytometry and ELISA.

Alternatively, or in addition, the ability of the cells to survive viral infection is determined, for example, by performing a cell viability assay, such as trypan blue exclusion.

The ability of an agent that inhibits a Rab9 modulator to prevent or decrease infection by a virus, such as HIV, Ebola, or influenza A, can be assessed in animal models. Several animal models for viral infection are known in the art. For example, mouse HIV models are disclosed in Sutton *et al.* (*Res. Initiat Treat. Action*, 8:22-4, 2003) and Pincus *et al.* (*AIDS Res. Hum. Retroviruses* 19:901-8, 2003); guinea pig models for Ebola infection are disclosed in Parren *et al.* (*J. Virol.* 76:6408-12, 2002) and Xu *et al.* (*Nat. Med.* 4:37-42, 1998); and cynomolgus monkey (*Macaca fascicularis*) models for influenza infection are disclosed in Kuiken *et al.* (*Vet. Pathol.* 40:304-10, 2003). Such animal models can also be used to test agents for an ability to ameliorate symptoms associated with viral infection. In addition, such animal models can be used to determine the LD50 and the ED50 in animal subjects, and such data can be used to determine the *in vivo* efficacy of potential agents.

Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, e.g., baboons, monkeys, and chimpanzees, can be used to generate an animal model of viral infection if needed.

In this model, the appropriate animal is inoculated with the desired virus, in the presence or absence of the agent that inhibits a Rab9 modulator. The amount of virus and agent administered can be determined by skilled practitioners. In some examples, several different doses of the potential therapeutic agent can be administered to different test subjects, to identify optimal dose ranges. The therapeutic agent can be administered before, during, or after infection with the virus. Subsequent to the treatment, animals are observed for the development of the appropriate viral infection and symptoms associated therewith. A decrease in the development of the appropriate viral infection, or symptoms associated therewith, in the presence of the agent provides

evidence that the agent is a therapeutic agent that can be used to decrease or even inhibit viral infection in a subject.

Methods of Inhibiting Viral Infection

Also provided by the present invention is a method of inhibiting viral infection in a cell comprising inhibiting a modulator of Rab9. Inhibition can occur, *in vitro*, *ex vivo* or *in vivo*. One or more Rab9 modulators involved in vesicle transport from endosomes to the trans-Golgi can be inhibited. These include, but are not limited to, mannose-6-phosphate receptor, mapmodulin, TSG101, Rab9 effector p40, Yip3, PIKfyve, Rab-GDI, GDF, TIP47 and mapmodulin, a v-SNARE protein, a T-SNARE protein and a guanine nucleotide exchange factor (GEF).

Antisense oligonucleotides, RNAi molecules, ribozymes and siRNA molecules that recognize a Rab9 modulator involved in viral infection can be utilized to disrupt expression of the Rab9 modulator. Antisense oligonucleotides, RNAi molecules, ribozymes and siRNA molecules can be used alone or in combination with other therapeutic agents such as anti-viral compounds.

A subject susceptible to or suffering from a viral infection can be treated with a therapeutically effective amount of an antisense oligonucleotide, an RNAi molecule, a ribozyme or an siRNA molecule (or combinations thereof) that recognizes a Rab9 modulator involved in viral infection. After the antisense oligonucleotide, an RNAi molecule, a ribozyme or an siRNA molecule has taken effect (a decreased level of viral infection is observed, or symptoms associated with viral infection decrease), for example after 24-48 hours, the subject can be monitored for symptoms associated with viral infection.

Similarly, other agents, such as an antibody, polypeptide, small molecule or other drug that recognizes a host protein involved in viral infection and prevents a Rab9 modulator from interacting with Rab9 or from interacting with a viral protein, can also be used to decrease or inhibit viral infection. After the antibody, polypeptide, small molecule or other drug has taken effect (a decreased level of viral infection is observed, or symptoms associated with viral infection decrease), for example after 24-48 hours, the subject can be monitored for symptoms associated with viral infection. Other agents such as peptides and organic or inorganic molecules can also be administerd to a subject in a therapeutically effective amount.

The treatments disclosed herein can also be used prophylactically, for example, to inhibit or prevent a viral infection. Such administration is indicated where the treatment is shown to have utility for treatment or prevention of the disorder. The prophylactic use is indicated in conditions known or suspected of progressing to disorders associated with viral infection.

Pharmaceutical Compositions and Modes of Administration

Various delivery systems for administering the therapies disclosed herein are known, and include encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (Wu and Wu, *J. Biol. Chem.* 1987, 262:4429-32), and construction of therapeutic nucleic acids as part of a retroviral or other vector. Methods of introduction include, but are not limited to, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The compounds can be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (for example, oral mucosa, rectal, vaginal and intestinal mucosa, etc.) and can be administered together with other biologically active agents. Administration can be systemic or local. Pharmaceutical compositions can be delivered locally to the area in need of treatment, for example by topical application.

Pharmaceutical compositions are disclosed that include a therapeutically effective amount of an RNA, DNA, antisense molecule, ribozyme, siRNA, molecule, specific-binding agent, or other therapeutic agent, alone or with a pharmaceutically acceptable carrier. Furthermore, the pharmaceutical compositions or methods of treatment can be administered in combination with (such as before, during, or following) other therapeutic treatments, such as other antiviral agents.

Delivery systems

The pharmaceutically acceptable carriers useful herein are conventional. *Remington's Pharmaceutical Sciences*, by Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the therapeutic agents herein disclosed. In general, the nature of the carrier will depend on the mode of administration being employed. For instance, parenteral formulations usually include injectable fluids that include

pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, sesame oil, glycerol, ethanol, combinations thereof, or the like, as a vehicle. The carrier and composition can be sterile, and the formulation suits the mode of administration. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. For solid compositions (for example powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, sodium saccharine, cellulose, magnesium carbonate, or magnesium stearate. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

Embodiments of the disclosure including medicaments can be prepared with conventional pharmaceutically acceptable carriers, adjuvants and counterions as would be known to those of skill in the art.

The amount of therapeutic agent effective in decreasing or inhibiting viral infection can depend on the nature of the virus and its associated disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays can be employed to identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The disclosure also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Instructions for use of the composition can also be included.

In an example in which a nucleic acid is employed to reduce viral infection, such as an antisense or siRNA molecule, the nucleic acid can be delivered intracellularly (for example by expression from a nucleic acid vector or by receptor-mediated mechanisms), or by an appropriate nucleic acid expression vector which is administered so that it becomes intracellular, for example by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (such as a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (for example Joliot *et al.*, *Proc. Natl. Acad. Sci. USA* 1991, 88:1864-8). The present disclosure includes all forms of nucleic acid delivery, including synthetic oligos, naked DNA, plasmid and viral delievery, integrated into the genome or not.

The present invention also provides a method of screening compounds for antiviral activity comprising contacting the compound with a cell expressing a Rab9 modulator and determining if viral infection is inhibited via the Rab9 modulator.

One of skill in the art can readily determine if viral infection is inhibited in the cell, as described herein. Furthermore, one of skill in the art can determine the levels of the Rab9 modulator and correlate these levels with the amount of viral infection. For example, if viral infection is inhibited and there is a decrease in the amount of the Rab9 modulator, the compound inhibits viral infection via the Rab9 modulator. The compounds identified utilizing these methods can be used to inhibit viral infection in cells either *in vitro*, *ex vivo* or *in vivo*.

The amount of Rab9 modulator protein in a cell can be determined by methods standard in the art for quantitating proteins in a cell, such as Western blotting, ELISA, ELISPOT, immunoprecipitation, immunofluorescence (e.g., FACS), immunohistochemistry, immunocytochemistry, etc., as well as any other method now known or later developed for quantitating protein in or produced by a cell.

The amount of Rab9 modulator nucleic acid in a cell can be determined by methods standard in the art for quantitating nucleic acid in a cell, such as *in situ* hybridization, quantitative PCR, RT-PCR, Northern blotting, ELISPOT, dot blotting, etc., as well as any other method now known or later developed for quantitating the amount of a nucleic acid in a cell.

Other methods of screening for agents that disrupt the interaction between 1) a Rab9 modulator and Rab9 or 2) a Rab9 modulator and a viral protein. For example,

yeast two-hybrid assays are well known in the art (see Chien et al., Proc. Natl. Acad. Sci. USA, 88:9578-82 (1991).

Transgenic Cells and Non-Human Mammals

Transgenic animal models, including recombinant and knock-out animals, can be generated from the host nucleic acids described herein. Exemplary transgenic non-human mammals include, but are not limited to, mice, rats, chickens, cows, and pigs. The present invention provides a transgenic non-human mammal has a knock-out of one or more RAb9 modulators and has a decreased susceptibility to infection by pathogens, such as viruses, bacteria and fungi. Such knock-out animals are useful for reducing the transmission of viruses from animals to humans.

Expression of the sequence used to knock-out or functionally delete the desired gene can be regulated by chosing the appropriate promoter sequence. For example, constitutive promoters can be used to ensure that the functionally deleted gene is not expressed by the animal. In contrast, an inducible promoter can be used to control when the transgenic animal does or does not express the gene of interest. Exemplary inducible promoters include tissue-specific promoters and promoters responsive or unresponsive to a particular stimulus (such as light, oxygen, chemical concentration, such as a tetracycline inducible promoter).

For example, a transgenic mouse including a Rab9 modulator gene or a mouse having a disrupted Rab9 modulator gene, can be examined during exposure to various pathogens. Comparison data can provide insight into the life cycles of pathogens. Moreover, knock-out animals (such as pigs) that are otherwise susceptible to an infection (for example influenza) can be made to determine the resistance to infection conferred by disruption of the gene.

Transgenic animals, including methods of making and using transgenic animals, are described in various patents and publications, such as WO 01/43540; WO 02/19811; U.S. Pub. Nos: 2001-0044937 and 2002-0066117; and U.S. Pat. Nos: 5,859,308; 6,281,408; and 6,376,743; and the references cited therein.

Cells including an altered or disrupted Rab9 modulator gene having a role in viral infection are resistant to infection by a pathogen. Such cells may therefore include cells having decreased susceptibility to HIV infection, Ebola infection, influenza A or any of the other pathogens described herein, including bacteria and fungi.

The present invention also provides a method of screening a cell for a variant form of Rab9 or a Rab9 modulator. These cells containing a variant form of Rab9 or a Rab9 modulator can be contacted with a pathogen to determine if cells comprising a naturally occurring variant of Rab9 and cells comprising naturally occurring variants of Rab9 modulators differ in their resistance to infection.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

What is claimed is:

1. A method of identifying a modulator of Rab9 that is involved in viral infection comprising:

   a) inhibiting the expression of a putative modulator of Rab9 in a cell that expresses Rab9;

   b) contacting the cell with a virus;

   c) detecting the level of viral infection, a decrease or elimination of viral infection indicating that the putative modulator of Rab9 is a modulator of Rab9 that is involved in viral infection.

2. A method of identifying a modulator of Rab9 that is involved in viral infection comprising:

   a) inhibiting the expression of a putative modulator of Rab9 in a cell that expresses Rab9;

   b) contacting the cell with a virus;

   c) detecting the level of viral infection; and

   d) associating the level of viral infection with the level of Rab9 and/or Rab9 activity, a decrease or elimination of viral infection associated with a decrease or elimination of Rab9 and/or Rab9 activity indicating that the putative modulator of Rab9 is a modulator of Rab9 that is involved in viral infection.

3. The method of claim 1 or 2, wherein decreasing expression of a putative modulator comprises knocking out the function of the putative modulator.

4. The method of claim 1 or 2, wherein decreasing expression of a putative modulator of Rab9 comprises decreasing or disrupting mRNA encoding the putative modulator of Rab9.

5. The method of claim 4, wherein decreasing or disrupting the mRNA comprises contacting the mRNA with an antisense RNA, RNAi, ribozyme, or siRNA that recognizes the mRNA.

6. The method of claim 1 or 2, wherein the modulator modulates Rab9 expression via transcriptional activation.

7. The method of claim 1 or 2, wherein the modulator modulates Rab9 activity by binding to Rab9.

8. The method of claim 1 or 2, wherein the modulator is a v-SNARE protein.

9. The method of claim 1 or 2, wherein the modulator is a t-SNARE protein.

10. The method of claim 1 or 2, wherein the modulator is a Rab9 effector.

11. The method of claim 10, wherein the Rab9 effector is p40.

12. The method of claim 1 or 2, wherein the modulator is a GDP dissociation inhibitor.

13. The method of claim 1 or 2, wherein the modulator is a guanine-nucleotide exchange factor (GEF).

14. The method of claim 1 or 2, wherein the modulator is a guanine-nucleotide displacement factor (GDF).

15. The method of claim 14, wherein the GDF is Yip3.

16. The method of claim 1 or 2, wherein the modulator is PIKfyve.

17. The method of claim 1 or 2, wherein the modulator is mannose-6-phosphate receptor.

18. The method of claim 1 or 2, wherein the modulator is a protein involved in trafficking proteins and other molecules from the late endosome to the trans-Golgi via interaction with Rab9.

19. A method of determining if a modulator of Rab9 is associated with viral infection comprising inhibiting the expression of the Rab9 modulator in a cell that expresses Rab9 and determining if viral infection is inhibited.

20. The method of claim 19, wherein inhibiting expression of the Rab9 modulator comprises knocking out the function of the modulator.

21. The method of claim 19, wherein inhibiting expression of the Rab9 modulator comprises decreasing or disrupting mRNA encoding the Rab9 modulator.

22. The method of claim 21, wherein decreasing mRNA comprises contacting the mRNA with an antisense RNA, RNAi, ribozyme, or siRNA that recognizes the mRNA.

23. The method of claim 19, wherein the modulator modulates Rab9 expression via transcriptional activation.

24. The method of claim 19, wherein the modulator modulates Rab9 activity by binding to Rab9.

25. The method of claim 19, wherein the modulator is a v-SNARE protein.

26. The method of claim 19, wherein the modulator is a t-SNARE protein.

27. The method of claim 19, wherein the modulator is a Rab9 effector.

28. The method of claim 27, wherein the Rab9 effector is p40.

29. The method of claim 19, wherein the modulator is a GDP dissociation inhibitor.

30. The method of claim 19, wherein the modulator is a guanine-nucleotide exchange factor (GEF).

31. The method of claim 19, wherein the modulator is a guanine-nucleotide displacement factor (GDF).

32. The method of claim 31, wherein the GDF is Yip3.

33. The method of claim 19, wherein the modulator is PIKfyve.

34. The method of claim 19, wherein the modulator is mannose-6-phosphate receptor.

35. The method of claim 19, wherein the modulator is a protein involved in trafficking proteins and other molecules from the late endosome to the trans-Golgi via interaction with Rab9.

36. A method of inhibiting viral infection in a cell comprising inhibiting a modulator of Rab9.

37. The method of claim 36, wherein inhibiting a modulator of Rab9 comprises knocking out the function of the modulator.

38. The method of claim 36, wherein inhibiting a modulator of Rab9 comprises decreasing or disrupting mRNA encoding the modulator of Rab9.

39. The method of claim 38, wherein decreasing mRNA comprises contacting the mRNA with an antisense RNA, RNAi, ribozyme, or siRNA that recognizes the mRNA.

40. The method of claim 36, wherein the modulator modulates Rab9 expression via transcriptional activation.

41. The method of claim 36, wherein the modulator modulates Rab9 activity by binding to Rab9.

42. The method of claim 36, wherein the modulator is a v-SNARE protein.

43. The method of claim 36, wherein the modulator is a t-SNARE protein.

44. The method of claim 36, wherein the modulator is a Rab9 effector.

45. The method of claim 44, wherein the Rab9 effector is p40.

46. The method of claim 36, wherein the modulator is a GDP dissociation inhibitor.

47. The method of claim 36, wherein the modulator is a guanine-nucleotide exchange factor (GEF).

48. The method of claim 36, wherein the modulator is a guanine-nucleotide displacement factor (GDF).

49. The method of claim 48, wherein the GDF is Yip3.

50. The method of claim 36, wherein the modulator is PIKfyve.

51. The method of claim 36, wherein the modulator is mannose-6-phosphate.

52. The method of claim 36, wherein the modulator is a protein involved in trafficking proteins and other molecules from the late endosome to the trans-Golgi via interaction with Rab9.

53. A method of screening compounds for antiviral activity comprising contacting the compound with a cell expressing a modulator of Rab9 and determining if viral infection is inhibited by inhibition of the modulator of Rab9.

54. The method of claim 53, wherein the modulator modulates Rab9 expression via transcriptional activation.

55. The method of claim 53, wherein the modulator modulates Rab9 activity by binding to Rab9.

56. The method of claim 53, wherein the modulator is a v-SNARE protein.

57. The method of claim 53, wherein the modulator is a t-SNARE protein.

58. The method of claim 53, wherein the modulator is a Rab9 effector.

59. The method of claim 58, wherein the Rab9 effector is p40.

60. The method of claim 53, wherein the modulator is a GDP dissociation inhibitor.

61. The method of claim 53, wherein the modulator is a guanine-nucleotide exchange factor (GEF).

62. The method of claim 53, wherein the modulator is a guanine-nucleotide displacement factor (GDF).

63. The method of claim 62, wherein the GDF is Yip3.

64. The method of claim 53, wherein the modulator is PIKfyve.

65. The method of claim 53, wherein the modulator is mannose-6-phosphate receptor.

66. The method of claim 53, wherein the modulator is a protein involved in trafficking proteins and other molecules from the late endosome to the trans-Golgi via interaction with Rab9.

ABSTRACT

The present invention relates to a cellular protein, Rab9, Rab9 nucleic acid sequences and Rab9 proteins encoded by these sequences that are involved in viral infection or are otherwise associated with the life cycle of a virus. The invention also relates to modulators of Rab9 that are involved in viral infection or are otherwise associated with the life cycle of a virus.